TRIBONECTINS

This application is a continuation-in-part of patent application U.S. Serial No. 09/298,970, filed on April 23, 1999, the entire contents of which is hereby incorporated by reference.

Background of the Invention

The invention relates to lubrication of mammalian joints.

Osteoarthritis (OA) is the one of the most common form of joint disease. Factors which contribute to the development of OA include a family history of OA, previous damage to the joint through injury or surgery, and age of the joint, i.e., "wear and tear" of the articulating surfaces of the joint. OA is very common in older age groups, but can affect children as well.

Current treatment is directed to relieving pain and other symptoms of OA, e.g., by administering analgesics and anti-inflammatory drugs. Other therapeutic approaches include viscosupplementation by administering hyaluronic acid and derivatives thereof to joint tissue to increase the viscosity of synovial fluid.

Summary of the Invention

The invention features a novel treatment for osteoarthritis and other degenerative joint diseases by tribosupplementation. Tribosupplementation is carried out by administering lubricating polypeptides directly to the injured or arthritic joint. Unlike viscosupplementation, tribosupplementation does not substantially increase the viscosity of the solution, e.g., synovial fluid, to which it is added. The viscosity of a solution to which a tribonectin is added increases no more than 10%, preferably no more than 5%, more preferably no more than 2%, more preferably no more than 1%. Most preferably, the viscosity of the solution to which a tribonectin is added is unaltered or decreases.

Accordingly, the invention provides a tribonectin. A tribonectin is an artificial boundary lubricant which contains at least one repeat of an amino acid sequence which is at least 50% identical to KEPAPTT (SEQ ID NO:3). A tribonectin comprising at least one O-linked lubricating moiety. Preferably the lubricating moiety is a β(1-3)Gal-GalNAc moiety. The amino acid sequence of the protein backbone of a naturally-occurring tribonectin may differ depending on alternative splicing of exons of the human megakaryocyte stimulating factor (MSF) gene. For example, the tribonectin contains amino acids 1 to 24 and 200 to 1404 of SEQ ID NO:1 but lacks

amino acids 25-199 of SEQ ID NO:1. The tribonectin contains amino acids 1 to 156 and 200 to 1404 of SEQ ID NO:1 but lacks amino acids 157 to199 of SEQ ID NO:1. The tribonectin contains amino acids 1 to 106 of SEQ ID NO:1 and 200 to 1404 of SEQ ID NO:1 but lacks acids 107 to 199 of SEQ ID NO:1. The tribonectin contains amino acids 1 to 25 of SEQ ID NO:1, 67 to 106 of SEQ ID NO:1 and 200-to 1404 of SEQ ID NO:1 but lacks amino acids 26 to 66 of SEQ ID NO:1. Tribonectins are purified naturally-occurring polypeptides or sythetically produced or recombinant polypeptides. The amino acid sequence of the backbone of synthetic or rcombinant tribonectins is at least 50% identical to the amino acid sequence of a naturally-occurring tribonectin and possess at least 50% of the lubricating activity of a naturally-occurring tribonectin.

A tribonectin is formulated for administration to a mammalian joint or other tissue, e.g., cardiac tissue, for which lubrication is desired. Preferably, the tribonectin is a recombinant or chemically-synthesized lubricating polypeptide. For example, a tribonectin includes a substantially pure polypeptide the amino acid sequence of which includes at least one repeats but less than 76 repeats. Each subunit contains at least 7 amino acids (and typically, 10 or fewer amino acids). The amino acid sequence of each subunit is at least 50% identical to SEQ ID NO:3, and a non-identical amino acid in the reference sequence is a conservative amino acid substitution. For example, one or both of the threonine residues are substituted with a serine residue. Preferably, the amino acid sequence of the subunit is identical to SEQ ID NO:3. The tribonectin may also contain one or more repeats of the amino acid sequence XXTTTX (SEQ ID NO:4). Polypeptides or other compounds described herein are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

Where a particular polypeptide or nucleic acid molecule is said to have a specific percent identity to a reference polypeptide or nucleic acid molecule of a defined length, the percent identity is relative to the reference polypeptide or nucleic acid molecule. Thus, a peptide that is 50% identical to a reference polypeptide that is 100 amino acids long can be a 50 amino acid polypeptide that is completely identical to a 50 amino acid long portion of the reference

polypeptide. It can also be a 100 amino acid long-polypeptide which is 50% identical to the reference polypeptide over its entire length.

A polypeptide or nucleic acid molecule which is "substantially identical" to a given reference polypeptide or nucleic acid molecule is a polypeptide or nucleic acid molecule having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference polypeptide sequence or nucleic acid molecule. "Identity" has an art-recognized meaning and is calculated using well known published techniques, e.g., Computational Molecular Biology, 1988, Lesk A.M., ed., Oxford University Press, New York; Biocomputing: Informatics and Genome Projects, 1993, Smith, D.W., ed., Academic Press, New York; Computer Analysis of Sequence Data, Part I, 1994, Griffin, A.M. and Griffin, E.G., eds., Eumana Press, New Jersey; Sequence Analysis in Molecular Biology, 1987, Heinje, G., Academic Press, New 20 York; and Sequence Analysis Primer, 1991, Gribskov, M. and Devereux, J., eds., Stockton Press, New York). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans and has a definite meaning with respect to a given specified method. Sequence identity described herein is measured using the Lasergene software package (DNASTAR, Inc. Madison, WI). The MegAlign module used is the Clustal V method (Higgins et al. 1989, CABIOS 5 (2):151-3. The parameters used are gap penalty 10, gap length penalty 10.

Alternatively, nucleic acids which differ from a given reference sequence hybridize at high stringency to a stand of DNA having the reference sequence or the complement thereof. High stringency conditions are hybridization at 42 degrees C in 50% formamide, a first wash at 65 degrees C in 2 X SSC and a second wash at 65 degrees C in 0.2 X SSC.

A tribonectin is characterized as reducing the coefficient of friction (μ) between bearing surfaces. For example, reduction of friction is measured *in vitro* by detecting a reduction in friction in a friction apparatus using latex:glass bearings. Reduction of friction is also measured *in vivo*, e.g., by measuring reduction of patient pain. Tribonectins of the invention are lubricating compositions. Polypeptides that have at least 50% (but less than 100%) amino acid sequence identity to a reference sequence are tested for lubricating function by measuring a reduction in the μ between bearing surfaces.

A tribonectin includes an O-linked oligosaccharide, e.g., an N-acetylgalactosamine and galactose in the form beta (l-3)Gal-GalNAC. For example, KEPAPTT (SEQ ID NO:3) and XXTTTX (SEQ ID NO:4) repeat domains are glycosylated by beta (l-3)Gal-GalNAC (which may at times be capped with NeuAc in the form of (l-3)Gal-GalNAC-NeuAc. The term "glycosylated" with respect to a polypeptide means that a carbohydrate moiety is present at one or more sites of the polypeptide molecule. For example, at least 10%, preferably at least 20%, more preferably at least 30%, and most preferably at least 40% of the tribonectin is glycosylated. Up to 50% or more of the tribonectin can be glycosylated. Per cent glycosylation is determined by weight.

A tribonectin polypeptide contains a substantially pure fragment of (MSF. For example, the molecular weight of a substantially pure tribonectin having an amino acid sequence of a naturally-occurring tribonectin is in the range of 220-280 kDa. Preferably, the apparent molecular weight of a tribonectin is less than 230 kDa, more preferably less than 250 kDa, and most preferably less than 280 kDa. A protein or polypeptide fragment is defined as a polypeptide which has an amino acid sequence that is identical to part, but not all, of the amino acid sequence of a naturally-occurring protein or polypeptide from which it is derived, e.g., MSF. The tribonectin may contain a polypeptide, the amino acid sequence of which is at least 50% identical to the sequence of residues 200-1140, inclusive, of SEQ ID NO:l, e.g., it contains the amino acid sequence of residues 200-1140, inclusive, of SEQ ID NO:l. In another example, the polypeptide contains an amino acid sequence that is at least 50% identical to the sequence of residues 200-1167, inclusive, of SEQ ID NO:l, e.g., one having the amino acid sequence identical to residues 200-1167, inclusive, of SEQ ID NO:1. The polypeptide contains an amino acid sequence that is at least 50% identical to the sequence of residues 200-1212, inclusive, of SEQ ID NO:l, e.g., the amino acid sequence of residues 200-1212, inclusive, of SEQ ID NO:l, or the polypeptide contains an amino acid sequence that is at least 50% identical to the sequence of residues 200-1263, inclusive, of SEQ ID NO:l, e.g., an amino acid sequence identical to residues 200-1263, inclusive, of SEQ ID NO:l. Preferably, the sequence of the polypeptide lacks the amino acid sequence of residues 1-24, inclusive, of SEQ ID NO:l and/or the amino acid sequence of residues 67-104, inclusive of SEQ ID NO:l.

The invention also features an isolated nucleic acid molecule encoding a tribonectin. For example, the nucleic acid includes a sequence that is at least 50% identical to nucleotides 631-

3453, inclusive, of SEQ ID NO:2. Preferably, the nucleic acid encodes a polypeptide with the amino acid sequence of residues 200-1140 of SEQ ID NO:1. For example, the nucleic acid has a nucleotide sequence identical to that of nucleotides 631-3453, inclusive, of SEQ ID NO:2, or a degenerate variant thereof. An isolated nucleic acid molecule is a nucleic acid molecule that is separated from the 5' and 3' coding sequences or non-coding sequences with which it is immediately contiguous in the naturally occurring genome of an organism. Isolated nucleic acid molecules include nucliic acid molecules which are not naturally occurring, e.g., nucleic acid molecules created by recombinant DNA techniques. For example, the nucleic acid of the invention includes nucleotides 631-3453, inclusive, of SEQ ID NO:2, but not nucleotides which are immediately contiguous to those sequences in the naturally-occurring genomic sequence or naturally-occurring cDNA.

Also within the invention is a method of lubricating a mammalian joint by contacting the joint with purified MSF or a tribonectin. The mammal is preferably a human, horse, dog, ox, donkey, mouse, rat, guinea pig, cow, sheep, pig, rabbit, monkey, or cat, and the joint is an articulating joint such as a knee, elbow, shoulder, hip, or any other weight-bearing joint. Tribonectins are administered intra-articularly. Therapeutic joint lubrication is also carried out by gene therapy, e.g., by contacting the joint or synovial fluid with a nucleic acid encoding a tribonectin. For example, nucleic acids are administered to a synovial cavity by intra-articular injection.

In addition to functioning as a boundary lubricant in a mammalian joint, a tribonectin is used as a boundary lubricant between soft mammalian tissues such as skin or internal organs or between a mammalian tissue and a medical device such as a prosthetic implant. Accordingly, the invention encompasses a biocompatible composition containing a tribonectin in a form suitable for the inhibition of tissue adhesion formation. For example, the tribonectin is in the form of a film, membrane, foam, gel, or fiber. The term "film," as used herein, means a substance formed by compressing a foam or gel to a thin membrane, e.g., by casting into a flat mold and air drying to a thin membrane, or by compressing a gel or fibers, or by allowing or causing a gel or fibers to dehydrate. The term "foam," as used herein, means a substance with a porous structure formed, e.g., by introduction of as air into a tribonectin solution, suspension, gels, or fiber. The term "bioabsorbable," as used herein, refers to the ability of a tissue-compatible material to degrade in the body after implantation, into nontoxic products which are

eliminated from the body or metabolized. A "biocompatible" substance, as the term is used herein, is one that has no medically unacceptable toxic or injurious effects on biological function. Tribonectin compositions for the prevention of adhesions are also formulated as compositions suitable for extrusion, e.g., to form a mold upon which tissue can grow without adhering.

A method inhibiting adhesion formation between a first surface and a second surface in a mammal is carried out by placing a tribonectin between the first and second surfaces in an amount sufficient to prevent adhesion of the surfaces in the mammal. For example, one or both of the surfaces is a mammalian tissue, and a tribonectin placed between them prevents formation of adhesions during the healing process. Alternatively the first or the second surface (or both) is an artificial device such as an orthopedic implant. Tissues to be treated include those injured by surgical incision or trauma.

Also within the invention is a method for diagnosing osteoarthritis or a predisposition thereto by obtaining a biological sample from a mammal and measuring the amount of an MSF fragment in the biological sample. An increase in the amount compared to a control, e.g., a predetermined value associated with a negative diagnosis or a biological sample from a mammal known to be free of osteoarthritis, indicates that the mammal suffers from osteoarthritis or is predisposed to developing osteoarthritis. Any biological sample is suitable for testing in the diagnostic method; typically, the biological sample is synovial fluid, blood, serum, or urine. Preferably, the MSF fragment contains the amino acid sequence of SEQ ID NO:3. Alternatively, the MSF fragment contains the amino acid sequence of EPAPTT (SEQ ID NO:5; a product of trypsin cleavage of a tribonectin) or the amino acid sequence of PTTKEP (SEQ ID NO:6; a product of elastase cleavage of a tribonectin).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Brief Description of the Drawings

Fig. 1 is a diagram of a sequence alignment of the amino acid sequence of MSF and a human tribonectin. MSF exons 7, 8, and 9 are shown in their entirety; exon 6 was abbreviated due to its length (940 amino acids). Exon 6 contains a total of 76 repeats of the degenerate sequence KEPAPPT (SEQ ID NO:3) which function as sites for O-glycosylation. C-terminal lysine/arginine residues of sequenced tryptic fragments are shadowed. Amino acids 214-222 and 300-309 correspnding to exon 6-specific forward and reverse primers are shadowed and

underlined. The amino acid sequence coordinates refer to the amino acid sequence of SEQ ID NO:1.

Figs. 2A-B are diagrams of phenotypic isoforms of tribonectin expression by human synovial fibroblasts and articular chondrocytes, respectively. Fig. 2A graphically shows MSF exons expressed in tribonectin isoforms. Fig. 2B schematically shows exon expression in articular chondrocytes compared to synovial fibroblasts Exons 2, 4, and 5 are alternatively spliced resulting in isoform expression.

Fig. 3 is a diagram showing the alternative splicing boundaries of tribonectin isoforms V1, V2, and V3.

Detailed Description

A number of naturally-occurring tribonectin isoforms wer identified and isolated. For example, a human lubricating polypeptide was purified from synovial fluid and found to contain amino acids encoded by exons 2 and 4-12 of the MSF gene (but not exons 1 or 3). The gene encoding naturally-occurring full length MSF contains 12 exons, and the naturally-occurring MSF gene product contains 1404 amino acids with multiple polypeptide sequence homologies to vitronectin including hemopexin-like and somatomedin-like regions. Centrally-located exon 6 contains 940 residues. Exon 6 encodes a O-glycosylated mucin domain. A polypeptide encoded by nucleotides 631-3453 of SEQ ID NO:2 provides boundary lubrication of articular cartilage.

Table 1: MSF amino acid sequence

MAWKTLPIYLLLLLSVFVIQQVSSQDLSSCAGRCGEGYSRDATCNCDYNCQHYMECCP
DFKRVCTAELSCKGRCFESFERGRECDCDAQCKKYDKCCPDYESFCAEVHNPTSPPSSK
KAPPPSGASQTIKSTTKRSPKPPNKKKTKKVIESEEITEEHSVSENQESSSSSSSSSSSTIW
KIKSSKNSAANRELQKKLKVKDNKKNRTKKKPTPKPPVVDEAGSGLDNGDFKVTTPDT
STTQHNKVSTSPKITTAKPINPRPSLPPNSDTSKETSLTVNKETTVETKETTTTNKQTSTD
GKEKTTSAKETQSIEKTSAKDLAPTSKVLAKPTPKAETTTKGPALTTPKEPTPTTPKEPAS
TTPKEPTPTTIKSAPTTPKEPAPTTTKSAPTTPKEPAPTTTKEPAPTTPKEPAPTTTKEPAPT
TTKSAPTTPKEPAPTTPKKPAPTTPKEPAPTTPKEPTPTTPKEPAPTTKEPAPTTPKEPAPT
APKKPAPTTPKEPAPTTPKEPAPTTTKEPSPTTPKEPAPTTTKSAPTTTKEPAPTTTKSAPT
TPKEPSPTTTKEPAPTTPKEPAPTTPKKPAPTTPKEPAPTTPKEPAPTTTKKPAPTAPKEPA
PTTPKETAPTTPKKLTPTTPEKLAPTTPEKPAPTTPEELAPTTPEEPTPTTPEEPAPTTPKAA
APNTPKEPAPTTPKEPAPTTPKEPAPTTPKETAPTTPKGTAPTTLKEPAPTTPKKPAPKEL
APTTTKEPTSTTSDKPAPTTPKGTAPTTPKEPAPTTPKEPAPTTPKGTAPTTLKEPAPTTPK
KPAPKELAPTTTKGPTSTTSDKPAPTTPKETAPTTPKEPAPTTPKKPAPTTPETPPPTTSEV
STPTTTKEPTTIHKSPDESTPELSAEPTPKALENSPKEPGVPTTKTPAATKPEMTTTAKDK

TTERDLRTTPETTTAAPKMTKETATTTEKTTESKITATTTQVTSTTTQDTTPFKITTLKTT
TLAPKVTTTKKTITTTEIMNKPEETAKPKDRATNSKATTPKPQKPTKAPKKPTSTKKPKT
MPRVRKPKTTPTPRKMTSTMPELNPTSRIAEAMLQTTTRPNQTPNSKLVEVNPKSEDAG
GAEGETPHMLLRPHVFMPEVTPDMDYLPRVPNQGIIINPMLSDETNICNGKPVDGLTTLR
NGTLVAFRGHYFWMLSPFSPPSPARRITEVWGIPSPIDTVFTRCNCEGKTFFFKDSQYWR
FTNDIKDAGYPKPIFKGFGGLTGQIVAALSTAKYKNWPESVYFFKRGGSIQQYIYKQEPV
QKCPGRRPALNYPVYGEMTQVRRRRFERAIGPSQTHTIRIQYSPARLAYQDKGVLHNEV
KVSILWRGLPNVVTSAISLPNIRKPDGYDYYAFSKDQYYNIDVPSRTARAITTRSGQTLS
KVWYNCP (SEQ ID NO:1)

Table 2: MSF cDNA

1	gcggccgcga	ctattcggta	cctgaaaaca	acgatggcat	ggaaaacact	tcccatttac
61	ctgttgttgc	tgctgtctgt	tttcgtgatt	cagcaagttt	catctcaaga	tttatcaagc
121	tgtgcaggga	gatgtgggga	agggtattct	agagatgcca	cctgcaactg	tgattataac
181	tgtcaacact	acatggagtg	ctgccctgat	ttcaagagag	tctgcactgc	ggagctttcc
241	tgtaaaggcc	gctgctttga	gtccttcgag	agagggaggg	agtgtgactg	cgacgcccaa
301	tgtaagaagt	atgacaagtg	ctgtcccgat	tatgagagtt	tctgtgcaga	agtgcataat
361	cccacatcac	caccatcttc	aaagaaagca	cctccacctt	caggagcatc	tcaaaccatc
421	aaatcaacaa	ccaaacgttc	acccaaacca	ccaaacaaga	agaagactaa	gaaagttata
481	gaatcagagg	aaataacaga	agaacattct	gtttctgaaa	atcaagagtc	ctcctcctcc
541	tcctcctctt	cctcttcttc	ttcaacaatt	tggaaaatca	agttttccaa	aaattcagct
				EXON 6		
601	gctaatagag	aattacagaa	gaaactcaaa	gtaaaagata	acaagaagaa	cagaactaaa
661	aagaaaccta	cccccaaacc	accagttgta	gatgaagctg	gaagtggatt	ggacaatggt
721	gacttcaagg	tcacaactcc	tgacacgtct	accacccaac	acaataaagt	cagcacatct
781	cccaagatca	caacagcaaa	accaataaat	cccagaccca	gtcttccacc	taattctgat
841	acatctaaag	agacgtcttt	gacagtgaat	aaagagacaa	cagttgaaac	taaagaaact
901	actacaacaa	ataaacagac	ttcaactgat	ggaaaagaga	agactacttc	cgctaaagag
961	acacaaagta	tagagaaaac	atctgctaaa	gatttagcac	ccacatctaa	agtgctggct
1021	aaacctacac	ccaaagctga	aactacaacc	aaaggccctg	ctctcaccac	tcccaaggag
1081	cccacgccca	ccactcccaa	ggagcctgca	tctaccacac	ccaaagagcc	cacacctacc
1141	accatcaagt	ctgcacccac	cacccccaag	gagcctgcac	ccaccaccac	caagtctgca
1201	cccaccactc	ccaaggagcc	tgaacccacc	accaccaagg	agcctgcacc	caccactccc
1261	aaggagcctg	cacccaccac	caccaaggag	cctgcaccca	ccaccaccaa	gtctgcaccc
1321	accactccca	aggagcctgc	acccaccacc	cccaagaagc	ctgccccaac	taccccaaag
1381	gagcctgcac	ccaccactcc	caaggagcgc	acacccacca	ctcccaagga	gcctgcaccc
1441	accaccaagg	agcctgcacc	caccactccc	aaagagcttg	cacccactgc	ccccaagaag
1501	cctgccccaa	ctacccccaa	ggagcctgca	cccaccactc	ccaaggagcc	tgcacccacc
1561	accaccaagg	agccttcacc	caccactccc	aaggagcctg	cacccaccac	caccaagctc
1621	gcacccacca	ctaccaagga	gcctgcaccc	accactacca	agtctgcacc	caccactccc
1681	aaggagcctt	cacccaccac	caccaaggag	cctgcaccca	ccactcccaa	ggagcctgca
1741	cccaccaccc	ccaagaagcc	tgccccaact	acccccaagg	agcctgcacc	caccactccc
1801	aaggaacctg	cacccaccac	caccaagaag	cctgcagcca	ccgctcccaa	agagcctgcc
1861	ccaactaccc	ccaaggagac	tgcacccacc	acccccaaga	agctcacgcc	caccaccccc

1921	gagaagctcg	cacccaccac	ccctgagaag	cccgcaccca	ccacccctga	ggagctcgca
1981	cccaccaccc	ctgaggagcc	cacacccacc	acccctgagg	agcctgctcc	caccactccc
2041	aaggcagcgg	ctcccaacac	ccctaaggag	cctgctccaa	ctacccctaa	ggagcctgct
2101	ccaactaccc	ctaaggagcc	tgctccaact	acccctaagg	agactgctcc	aactaccct
2161	aaagggactg	ctccaactac	cctcaaggaa	cctgcaccca	ctactcccaa	gaagcctgcc
2221	tccaaggagc	ttgcacccac	caccaccaag	gagcccacat	ccaccacctc	tgacaagccc
2281	gctccaacta	cccctaaggg	gactgctcca	actaccccta	adgadcctgc	tccaactacc
2341	cctaaggagc	ctgctccaac	tacccctaag	gggactgctc	caactaccct	caaggaacct
2401	gcacccacta	ctcccaagaa	gcctgccccc	aaggagcttg	cacccaccac	caccaagggg
2461	cccacatcca	ccacctctga	caagcctgct	ccaactacac	ctaaggagac	tgctccaact
2521	acccccaagg	agcctgcacc	cactaccccc	aagaagcctg	ctccaactac	tcctgagaca
2581	cctcctccaa	ccacttcaga	ggtctctact	ccaactacca	ccaaggagcc	taccactatc
2641	cacaaaagcc	ctgatgaatc	aactcctgag	ctttctgcag	aacccacacc	aaaagctctt
2701	gaaaacagtc	ccaaggaacc	tggtgtacct	acaactaaga	ctcctgcagc	gactaaacct
2761	gaaatgacta	caacagctaa	agacaagaca	acagaaagag	acttacgtac	tacacctgaa
2821	actacaactg	ctgcacctaa	gatgacaaaa	gagacagcaa	ctacaacaga	aaaaactacc
2881	gaatccaaaa	taacagctac	aaccacacaa	gtaacatcta	ccacaactca	agataccaca
2941	ccattcaaaa	ttactactct	taaaacaact	actcttgcac	ccaaagtaac	tacaacaaaa
3001	aagacaatta	ctaccactga	gattatgaac	aaacctgaag	aaacagctaa	accaaaagac
3061	agagctacta	attctaaagc	gacaactcct	aaacctcaaa	agccaaccaa	agcacccaaa
3121	aaacccactt	ctaccaaaaa	gccaaaaaca	atgctcagag	tgagaaaacc	aaagacgaca
3181	ccaactcccc	gcaagatgac	atcaacaatg	ccagaattga	aaccctacctc	aagaatagca
3241	gaagccatgc	tccaaaccac	caccagacct	aaccaaactc	caaactccaa	actagttgaa
3301	gtaaatccaa	agagtgaaga	tgcaggtggt	gctgaaggag	aaacacctca	tatgcttctc
3361	aggccccatg	tgttcatgcc	tgaagttact	cccgacatgg	attacttacc	gagagtaccc
3421	aatcaaggca	ttatcatcaa	tcccatgctt	tccgatgaga	ccaatatatg	ccatggtaag
3481	ccagtagatg	gactgactac	tttgcgcaat	gggacattag	ttgcattccg	aggtcattat
3541	ttctggatgc	taagtccatt	cagtccacca	tctccagctc	gcagaattac	tgaagtttgg
3601	ggtattcctt	cccccattga	tactgttttt	actaggtgca	actgtgaagg	aaaaactttc
3661	ttctttaagg	attctcagta	ctggcgtttt	accaatgata	taaaagatgc	agggtacccc
3721	aaaccaattt	tcaaaggatt	tggaggacta	actggacaaa	tagtggcagc	gctttcaaca
3781	gctaaatata	agaactggcc	tgaatctgtg	tatttttca	agagaggtgg	cagcattcag
3841	cagtatattt	ataaacagga	acctgtacag	aagtgccctg	gaagaaggcc	tgctctaaat
3901	tatccagtgt	atggagaaat	gacacaggtt	aggagacgtc	gctttgaacg	tgctatagga
3961	ccttctcaaa	cacacaccat	cagaattcaa	tattcacctg	ccagactggc	ttatcaagac
4021	aaaggtgtcc	ttcataatga	agttaaagtg	agtatactgt	ggagaggact	tccaaatgtg
4081	gttacctcag	ctatatcact	gcccaacatc	agaaaacctg	acggctatga	ttactatgcc
4141	ttttctaaag	atcaatacta	taacattgat	gtgcctagta	gaacagcaag	agcaattact
4201	actcgttctg	ggcagacctt	atccaaagtc	tggtacaact	gtccttagac	tgatgagcaa
4261	aggaggagtc	aactaatgaa	gaaatgaata	ataaattttg	acactgaaaa	acattttatt
4321	aataaagaat	attgacatga	gtataccagt	ttatatataa	aaatgttttt	aaacttgaca
4381	atcattacac	taaaacagat	ttgataatct	tattcacagt	tgttattgtt	tacagaccat
4441	ttaattaata	tttcctctgt	ttattcctcc	tctccctccc	attgcatggc	tcacacctgt
4501	aaaagaaaaa	agaatcaaat	tgaatatatc	ttttaagaat	tcaaaactag	tgtattcact
4561	taccctagtt	cattataaaa	aatatctagg	cattgtggat	ataaaactgt	tgggtattct
4621	acaacttcaa	tggaaattat	tacaagcaga	ttaatccctc	tttttgtgac	acaagtacaa

4681	tctaaaagtt	atattggaaa	acatggaaat	attaaaattt	tacactttta	ctagctaaaa
4741	cataatcaca	aagctttatc	gtgttgtata	aaaaaattaa	caatataatg	gcaataggta
4801	gagatacaac	aaatgaatat	aacactataa	cacttcatat	tttccaaatc	ttaatttgga
4861	tttaaggaag	aaatcaataa	atataaaata	taagcacata	tttattatat	atctaaggta
4921	tacaaatctg	tctacatgaa	gtttacagat	tggtaaatat	catctgctca	acatgtaatt
4981	atttaataaa	actttggaac	attaaaaaaa	taaattggag	gcttaaaaaa	aaaaaaaaaa
5041	a (SEQ ID NO:2)					

Table 3: MSF Exon Boundaries

Exon	Amino acid sequence in SEQ ID NO:1	Nucleotide sequence in SEQ ID NO:2
1	1-24, inclusive	34-105, inclusive
2	25-66, inclusive	106-231, inclusive
3	67-104, inclusive	232-345, inclusive
4	105-155, inclusive	346-498, inclusive
5	156-199, inclusive	499-630, inclusive
6	200-1140, inclusive	631-3453, inclusive
7	1141-1167, inclusive	3454-3534, inclusive
8	1168-1212, inclusive	3535-3670, inclusive
9	1213-1263, inclusive	3671-3822, inclusive
10	1264-1331, inclusive	3823-4026, inclusive
11	1332-1371, inclusive	4027-4146, inclusive
12	1372-1404, inclusive	4147-4245, inclusive

The boundary lubricant isolated from synovial fluid is an alternatively-spliced variant of MSF. This alternatively-spliced variant was found to be the composition present in synovial fluid that confers lubricating capabilities to the articular joint. The boundary lubricant isolated from human synovial fluid contains amino acids encoded by exons 2, and 4-12 of the MSF gene, i.e., the alternative splice variant lacks amino acids encoded by exons 1 and 3 of the MSF gene. A recombinant or chemically-produced polypeptide containing at least exon 6 (but not exons 1 or 3) of MSF is useful to prevent and/or treat osteoarthritic disease. A recombinant or chemically-produced lubricating polypeptide containing at least one repeat of the amino acid sequence KEPAPTT (SEQ ID NO:3) either identically or with conservative substitution is also administered to lubricate mammalian joints.

Production and Purification of Recombinant Lubricating Polypeptides

To produce recombinant polypeptides, DNA containing exon 6 of MSF (nucleotides 631-3453 of SEQ ID NO:2) in an appropriate expression vector is transfected into a cell. The DNA can also contain some or all of exon 7 (nucleotides 354-3534 of SEQ ID NO:2) , exon 8 (nucleotides 3535-3670 of SEQ ID NO:2), or exon 9 (nucleotides 3671-3822 of SEQ ID NO:2) of the MSF gene. Primers for polymerase chain reaction (PCR) methods to generate DNA which spans various exons of MSF are shown below. Other isoforms are expressed by cloning nucleic acids corresponding to the exons of MSF encoding amino acid sequences sought to be expressed.

Table 4: PCR Primers

MSF exons	Forward Primer	Reverse Primer
Exon 2	5'AGATTTATCAAGCTGTGCAGGGAG3' (SEQ ID NO:7)	5'TTTACAGGAAAGCTCCGCAGTG3' (SEQ ID NO:8)
Exon 6	5'TCAAGGTCACAACTCCTGACACG3' (SEQ ID NO:9)	5'CTCTCGGTAAGTAATCCATGTCGG3' (SEQ ID NO:10)
Exons 2-12	5'TTGTTGCTGCTGTCTGTTTTCG3' (SEQ ID NO:11)	5'TGGATAAGGTCTGCCCAGAACGAG3' (SEQ ID NO:12)
Exons 6-12	5'TCAAGGTCACAACTCCTGACACG3' (SEQ ID NO: 13)	5'GATGGTGTGTGTTTGAGAAGGTCC3' (SEQ ID NO:14)

Methods of designing forward and reverse primers used to make DNAs which encode tribonectin polypeptides of varying lengths and which incorporate various exons of the MSF gene, e.g., to make polypeptide encoded by exons 2, 4-12; exons 6-9; and exons 2, 4-9, are well known in the art of molecular biology. Standard methods for transfecting cells with isolated nucleic acid are well known to those skilled in the art of molecular biology. For example, prokaryotic or eukaryotic cells in culture are transfected with the DNA of the invention operatively linked to expression control sequences appropriate for high-level expression in the cell. Such cells are useful for producing large amounts of the lubricating polypeptide, which are purified using

standard methods. The lubricating polypeptides are used therapeutically for treatment or prevention of arthritic diseases. The polypeptides are also used to raise antibodies against a naturally-occurring or recombinantly produced lubricating glycoproteins or glycopeptides.

For example, the recombinant gene product is expressed as a fusion protein and purified using a commercially available expression and purification system, e.g., the pFLAG expression system (IBI). The expression systems that may be used for purposes of the invention include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli and B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the nucleic acid molecules described herein. For production of glycosylated polypeptides, eukaryotic expression systems are used. Yeast (for example, Saccharomyces and Pichia) transformed with recombinant yeast expression vectors containing the recombinant nucleic acid encoding a tribonectin polypeptide are used. Insect cell systems infected with recombinant virus expression vectors (for example, baculovirus) containing the nucleic acid molecules encoding a tribonectin and mammalian cell systems (e.g., COS, CEO, BEK, 293, VERO, HeLa, MDCK, W138, and NIH 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., the metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter and the vaccinia virus 7.5K promoter) are also useful. In addition to clinical applications, recombinant polypeptides are injected into a rabbit or rodent to produce antibodies as described below.

The synovial fluid of an inflamed or injured joint contains proteolytic enzymes that degrade lubricating proteins or polypeptides. For example, infiltrating immune cells such as neutrophils secrete trypsin and/or elastase. Even a minor injury to an articulating joint or an inflammatory state can result in cellular infiltration and proteolytic enzyme secretion resulting in traumatic synovitis. Synovitis for a period of a few days or weeks can result in the loss of the cytoprotective layer of a joint, which in turn leads to the loss of cartilage. Non-lubricated cartilaginous bearings may experience premature wear which may initiate osteoarthritis. Individuals who clinically present with a traumatic effusion (e.g., "water on the knee") are predisposed to developing osteoarthritis; the elaboration of proteolytic enzymes degrades and depletes naturally-occurring lubricating compositions in the synovial fluid. Depletion of natural lubricating compositions occurs in other inflammatory joint diseases such as rheumatoid arthritis. Replacing or supplementing the synovial fluid of such injured joints with the lubricating

compositions of the invention prevents the development of osteoarthritis in the long term (e.g., years, even decades later) and immediately lubricates the joint to minimize short term damage.

Analogs, homologs, or mimetics of lubricating peptides which are less susceptible to degradation in vivo are used to lubricate mammalian joints. Analogs can differ from the naturally-occurring peptides by amino acid sequence, or by modifications which do not affect the sequence, or both. Modifications (which do not normally alter primary sequence) include *in vivo* or *in vitro* chemical derivitization of polypeptides, e.g., acetylation or carboxylation. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis-and processing or in further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian glycosylating or deglycosylating enzymes.

Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic bond renders the resulting peptide more stable, and thus more useful as a therapeutic. To render the therapeutic peptides less susceptible to cleavage by peptidases such as trypsin or elastise, the peptide bonds of a peptide may be replaced with an alternative type of covalent bond (a "peptide mimetic"). Trypsin, elastase, and other enzymes may be elaborated by infiltrating immune cells during joint inflammation. Trypsin cleaves a polypeptide bond on the carboxy-side of lysine and arginine; elastase cleaves on the carboxy-side of alanine, glycine. Thrombin, a serine protease which is present in hemorrhagic joints, cleaves a peptide bond on the carboxy-side of arginine. Collagenases are a family of enzymes produced by fibroblasts and chondrocytes when synovial metabolism is altered (e.g., during injury). These enzymes cut on the carboxy-side of glycine and proline. One or more peptidase-susceptible peptide bonds, e.g., those which appear in the KEPAPTT (SEQ ID NO:3) repeat sequence, are altered (e.g., replaced with a non-peptide bond) to make the site less susceptible to cleavage, thus increasing the clinical half-life of the therapeutic formulation.

Such mimetics, and methods of incorporating them into polypeptides, are well known in the art. Similarly, the replacement of an L-amino acid residue with a D-amino acid is a standard way of rendering the polypeptide less sensitive to proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl,

adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4, -dinitrophenyl.

Clinical formulations of tribonectins may also contain peptidase inhibitors such as N-methoxysuccinyl-AlaAla-Pro-Val chloromethylketone (an inhibitor of elastase).

Other clinically acceptable protease inhibitors (e.g., as described in Berling et al., 1998, Int. J. Pancreatology 24:9-17) such as leupeptin, aprotinin, α1-antitrypsin, α2-macroglobulin, α1-protease inhibitor, antichymotrypsin (ACHY), secretory leukocyte protease inhibitor (PSTI) are also co-administered with a tribonectin to reduce proteolytic cleavage and increase clinical halflife. A cocktail of two or more protease inhibitors can also be coadministered.

Compositions of tribonectin polypeptides or nucleic acids encoding the polypeptides are formulated in standard physiologically-compatible excipients known in the art., e.g., phosphate-buffered saline (PBS). Other formulations and methods for making such formulations are well known and can be found in, e.g., "Remington's Pharmaceutical Sciences". Tribonectins are also formulated with noncrosslinked hyaluronic acid preparations or viscosupplementation compositions, such as cross-linked hyaluronic acid preparations. When a tribonectin is added to a viscosupplement formulation, the- interaction of the tribonectin with hyaluronic acid reduces the viscosity of the viscosupplement.

Methods of making a glycopeptide and determining % glycosylation are known in the art, e.g., as described in U.S. Pat. No. 5,767,254. The presence of N-acetylgalactosamine is indicative of the presence of O-linked oligosaccharides (or Ser/Thr-linked oligosaccharides) in which GalNAc is commonly found in O-glycosidic alpha-linkage directly to amino acid. The presence of O-linked oligosaccharide is also detected by binding to Jacalin-Sepharose, an immobilized plant lectin that binds to the core disaccharide sequence Gal beta (1-3) GalNAc linked to Ser/Thr in glycoproteins, or peanut agglutinin, which binds to beta (1-3) Gal-GalNAC. O-linked oligosaccharides are distinguished from N-linked oligosacharides using standard methods. For example, oligosaccharides in O-glycosidic linkage, but not in N-glycosidic linkage, are susceptible to release from peptide by treatment with mild base in the presence of sodium borohydride (50 mM NaOH, 1M $NaBH_4$, 16 hr at 45°C.) to cause a beta-elimination reaction.

Veterinary Applications

Canine osteoarthritis is a prevalent clinical disorder that is treated using the methods described herein. Osteoarthritis afflicts an estimated one in five adult dogs; an estimated 8 million dogs suffer from this degenerative, potentially debilitating disease. Yet, many owners do not recognize the signs of chronic canine pain. While any dog can develop osteoarthritis, those most at risk are large breeds, geriatric dogs, very active dogs (such as working or sporting animals), and those with inherited joint abnormalities such as hip or elbow dysplasia.

Equine degenerative joint disease such as osteoarthritis is a cause of lameness and impaired performance in horses. As with humans and other mammals, degenerative joint diseases which affect horses are progressive disorders of synovial joints characterized by articular cartilage degeneration and joint effusion. Acute or chronic trauma, overuse, developmental disease, joint instability and old age leads to synovitis, impaired chondrocyte metabolism, and the formation of fissures in the joint cartilage. Destructive enzymes such as trypsin, elastase, stromelysin and hyaluronidase are released into the joint where they degrade synovial fluid and cartilage components, resulting in decreased synovial fluid viscosity, poor lubrication, depressed cartilage metabolism and enhanced wear resulting in pain and cartilage erosion. Current therapeutic approaches include medications for pain relief and anti-inflammatory drugs. The compositions and methods described herein are useful to replenish the lubricating capabilities of the affected joint.

Administration of Therapeutic Polypeptides

Standard methods for delivery of peptides are used. Such methods are well known to those of ordinary skill in the art. For intra-articular administration, tribonectin is delivered to the synovial cavity at a concentration in the range of 20-500 μg/ml in a volume of approximately 0.1-2 ml per injection. For example, 1 ml of a tribonectin at a concentration of 250 g/ml is injected into a knee joint using a fine (e.g., 14-22 gauge, preferably 18-22 gauge) needle. The compositions of the invention are also useful for parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal.

For prevention of surgical adhesions, the tribonectins described herein are administered in the form of gel, foam, fiber or fabric. A tribonectin formulated in such a manner is placed over and between damaged or exposed tissue interfaces in order to prevent adhesion formation between apposing surfaces. To be effective, the gel or film must remain in place and prevent

tissue contact for a long enough time so that when the gel finally disperses and the tissues do come into contact, they will no longer have a tendency to adhere. Tribonectins formulated for inhibition or prevention of adhesion formation (e.g., in the form of a membrane, fabric, foam, or gel) are evaluated for prevention of post-surgical adhesions in a rat cecal abrasion model (Goldberg et al., In Gynecologic Surgery and Adhesion Prevention. Willey-Liss, pp. 191-204, 1993). Compositions are placed around surgically abraded rat ceca, and compared to non-treated controls (animals whose ceca were abraded but did not receive any treatment). A reduction in the amount of adhesion formation in the rat model in the presence of the tribonectin formulation compared to the amount in the absence of the formulation indicates that the formulation is clinically effective to reduce tissue adhesion formation.

Tribonectins are also used to coat artificial limbs and joints prior to implantation into a mammal. For example, such devices are dipped or bathed in a solution of a tribonectin, e.g., as described in U.S. Pat. Nos. 5,709,020 or 5,702,456.

Lubricating polypeptides are at least about 10 amino acids (containing at least one KEPAPTT (SEQ ID NO:3) or at least one XXTTTX (SEQ ID NO:4) repeat), usually about 20 contiguous amino acids, preferably at least 40 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least about 60 to 80 contiguous amino acids in length. For example, the polypeptide is approximately 500 amino acids in length and contains 76 repeats of KEPAPTT (SEQ ID NO:3) . The polypeptide is less than 1404 residues in length, e.g., it has the amino acid sequence of naturally-occurring MSF (SEQ ID NO:l) but lacks at least 5, 10, 15, 20, or 24 amino acids of naturally-occurring MSF. Such peptides are generated by methods known to those skilled in the art, including proteolytic cleavage of a recombinant MSF protein, de novo synthesis, or genetic engineering, e.g., cloning and expression of at least exon 6, 7, 8, and/or 9 of the MSF gene.

Tribonectin polypeptides are also biochemically purified. The enzyme chymotrypsin cleaves at sites which bracket amino acids encoded by exon 6 of the MSF gene. Thus, a polypeptide containing amino acids encoded by exon of the MSF gene (but not any other MSF exons) is prepared from a naturally-occurring or recombinantly produced MSF gene product by enzymatic digestion with chymotrypsin. The polypeptide is then subjected to standard biochemical purification methods to yield a substantially pure polypeptide suitable for therapeutic administration, evaluation of lubricating activity, or antibody production.

Therapeutic compositions are administered in a pharmaceutically acceptable carrier (e.g., physiological saline). Carriers are selected on the basis of mode and route of administration and standard pharmaceutical practice. A therapeutically effective amount of a therapeutic composition (e.g., lubricating polypeptide) is an amount which is capable of producing a medically desirable result, e.g., boundary lubrication of a mammalian joint, in a treated animal. A medically desirable result is a reduction in pain (measured, e.g., using a visual analog pain scale described in Peyron et al., 1993, J. Rheumatol.(suppl.39) :10-15) or increased ability to move the joint (measured, e.g., using pedometry as described in Belcher et 30 al., 1997, J. Orthop. Trauma 11:106-109). Another method to measure lubricity of synovial fluid after treatment is to reaspirate a small volume of synovial fluid from the affected joint and test the lubricating properties in vitro using a friction apparatus as described herein.

As is well known in the medical arts, dosage for any one animal depends on many factors, including the animal's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Administration is generally local to an injured or inflamed joint. Alternatively, the polypeptides are administered via a timed-release implant placed in close proximity to a joint for slow release at the site of an injured or inflamed joint.

Gene Therapy

Gene therapy is carried out by administering to a mammal a nucleic acid encoding a therapeutic lubricating polypeptide, e.g., DNA encoding one or more repeats or the amino acid sequence KEPAPTT (SEQ ID NO:3) or DNA encoding a lubricating fragment of MSF, by standard vectors and/or gene delivery systems. Suitable gene delivery systems include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenoviruses and adeno-associated viruses.

In addition to a gene delivery system as described above, the therapeutic composition may include a pharmaceutically acceptable carrier, e.g., a biologically compatible vehicle such as physiological saline, suitable for administration to an animal. A therapeutically effective amount of a nucleic acid or polypeptide composition is an amount which is capable of producing a medically desirable result in a treated animal, e.g., a reduction in pain associated with joint movement, an increase in lubricating function of synovial fluid.

Parenteral administration, such as intravenous, subcutaneous, intramuscular, and intraperitoneal delivery routes, may be used to deliver the compound. Preferably, therapeutic compositions such as nucleic acids or polypeptides are delivered intra-articularly. Dosage for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs (e.g., anti-inflammatory drugs, viscotherapeutic drugs) being administered concurrently. A preferred dosage for administration of nucleic acids is from approximately 106 to 1022 copies of the nucleic acid molecule.

DNA is be introduced into target cells of the patient by standard vectors, e.g., a vector which contains DNA encoding a tribonectin operably linked to a promoter sequence. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, and adenoviruses, among others.

DNA may be administered locally using an adenovirus or adeno-associate virus delivery system using standard methods. For example, methods of delivering DNA intraarticularly to synovial fluid and methods of delivering DNA to cells from synovial fluid (e.g., synovial fibroblasts or chondrocytes) are described in U.S. Pat. No. 5,858,355. The only cis-acting sequences required for replication and packaging of recombinant adeno-associated virus (AAV) vector are the AAV terminal repeats. Up to 4 kb of DNA is inserted between the terminal repeats without effecting viral replication or packaging. To package a recombinant AAV vector, a plasmid containing the terminal repeats and DNA encoding a therapeutic polypeptide is co-transfected into cells with a plasmid that expresses AAV rep and capsid proteins. The transfected cells are then infected with adeno-associated virus, and recombinant AAV virus containing the desired sequences is isolated from cells approximately 48-72 hours after transfection. Recombinant virus is then administered for gene therapy applications using known methods.

Electroporation is another method of introducing DNA into target cells, e.g., synovial fibroblasts or chondrocytes, ex vivo. Cells to be electroporated are placed into Hepes buffer saline (EBS) at a concentration of about 10^7 cells per ml. The DNA to be electroporated is added at a concentration of approximately 5-20 micrograms/ml of HBS. The mixture is placed into an electroporation device and an electric field is applied according to standard protocols, e.g., in a range of between about 250 and 300 volts. Following introduction of DNA into synovial cells ex

vivo, the genetically modified autologous synovial cells are transplanted back into the donor by intra-articular injection. Approximately 10^7 cells are injected intra-articularly into joints in a volume of approximately 1 ml.

Synovial cells into which DNA is introduced are obtained using routine methods, e.g., through an arthroscope. The arthroscope is a small, hollow rod inserted into the knee via a small puncture wound which allows access to a surgical instrument to recover synovial cells arthroscopically. In some cases, the synovial cells in arthroscopically excised tissue are aseptically recovered by enzymatic digestion of the connective tissue matrix. For example, the synovium is cut into pieces of approximately 1 mm diameter and digested sequentially with trypsin (0.2% w/v in Gey's Balanced Salt Solution) for 30 minutes at 37°C, and collagenase (0.2% w/v in Gey's Balanced Salt Solution) for 2 hours at 37°C. A suspension of genetically-modified cells is injected into a recipient mammalian joint. Intra-articular injections of this type are routine and carried out in the doctor's office without additional surgical intervention. Repeat injections are carried out as needed.

Alternatively, the DNA (naked or packaged in a virus) is formulated in a suitable pharmaceutical carrier and injected intra-articularly. Gene therapy is also administered as a prophylactic measure to prevent the development of osteoarthritis in those individuals determined to be highly susceptible of developing this disease, e.g., those who have suffered an acute joint injury. Direct intra-articular injection of a DNA encoding a therapeutic polypeptide into a joint results in transfection of the recipient synovial cells to allow expression of DNA.

Drugs which stimulate an endogenous tribonectin promoter, e.g., TGF-beta, may also be administered as described above to increase the level of synovial expression.

Production of Antibodies Specific for Synovial Lubricating Polypeptides

Antibodies specific for lubricating polypeptides are obtained by techniques well known in the art. Such antibodies can be polyclonal or monoclonal. Polyclonal antibodies can be obtained, for example, by the methods described in Ghose et al., Methods in Enzymology, Vol. 93, 326-327, 1983. For example, a lubricating polypeptide encoded by nucleotides 632-3453 of SEQ ID NO:2 is used as an immunogen to stimulate the production of polyclonal antibodies in the antisera of a rabbit. Similar methods can be used to raise antisera in animals such as goats, sheep, and rodents.

Monoclonal antibodies are obtained by the well known process described by Milstein and Kohler in Nature, 256:495-497, 1975, or as modified by Gerhard, Monoclonal Antibodies, Plenum Press, 1980, pages 370-371. Hybridomas are screened to identify those producing antibodies that are highly specific for a synovial lubricating polypeptide. Preferably, the antibody has an affinity of at least about 10^8 liters/mole and more preferably, an affinity of at least about 10^9 liters/mole. Monoclonal or polyclonal antibodies provide a means of rapidly purifying large quantities of recombinant lubricating polypeptides.

In addition to antibodies which are directed to the peptide core of a tribonectin, an antibody directed to a sugar portion or to a glycopeptide complex of a tribonectin is desirable. To generate an antibody to the peptide core, a peptide spanning amino acids 200-350 of SEQ ID NO:1 is used. Shorter peptides, e.g., 8-15 amino acids in length, which are identical to an 8-15 amino acid portion of amino acids 200-350 of SEQ ID NO:1 are also used to generate such antibodies. Other peptides to be used as immunogens for antibodies specific for the peptide core of a tribonectin include those which are in the region of amino acids 24-66 of SEQ ID NO:1, amino acids 105-155 of SEQ ID NO:1, or amino acids 156-199 of SEQ ID NO:1. To generate antibodies which bind to a glycosylated tribonectin polypeptide (but not a deglycosylated or nonglycosylated form), the immunogen is preferably a glycopeptide, the amino acid sequence of which spans a highly glycosylated portion of a tribonectin, e.g., a peptide with an amino acid sequence of residues 200-1140 of SEQ ID NO:1. Shorter glycopeptides, e.g., 8-15 amino acids in length, within the same highly glycosylated region are also used as immunogens. Methods of generating antibodies to highly glycosylated biomolecules are known in the art, e.g., as described by Schneerson et al., 1980, J. Exp. Med. 152:361-376.

Methods of Diagnosis

Osteoarthritis is a disease that develops slowly and is difficult to diagnose until its late stages when joint pain often compels an individual to seek medical treatment. Early diagnosis of osteoarthritis or a predisposition to develop the disease allows early intervention to prevent or reduce the development of advanced osteoarthritis. The invention provides methods of early detection of this disease or a predisposition to develop it by tasting bodily fluids such as serum or urine for the presence of fragments of naturally-occurring tribonectins or the presence of fragments of MSF. Detection and quantitation of such peptides in biological fluids is well known in the art. For example, a standard sandwich ELISA assay is carried out using two

different antibodies (e.g., a first antibody which binds to an oligosaccharide portion of the glycopeptide and a second antibody which binds to the peptide core of the glycopeptide) to a naturally-occurring tribonectin. Alternatively, standard protein sequencing by liquid chromatography and mass spectroscopy, as is described below, is used to detect MSF fragments in biological samples. A control value is a predetermined value associated with a negative diagnosis; alternatively, a control sample is a biological sample from a mammal known to be free of osteoarthritis. An increase in the amount compared to a 30 control value or sample indicates that the mammal suffers from osteoarthritis or is predisposed to developing osteoarthritis.

Characterization of a Tribonectin from Human Synovial Fluid

Aliquots of synovial fluid from patients undergoing diagnostic arthroscopy and total knee replacement were collected and assayed in the friction apparatus. In both cases, the synovial fluid was aspirated prior to initiation of any surgical procedure and immediately centrifuged at 10,000 x g at 4°C for 2 hrs to remove cellular debris. Samples which were contaminated with blood were discarded. Aliquots with normal lubricating ability were pooled and stored at -20°C.

Purification and Isolation of a Tribonectin

Human synovial fluid (200 ml) was filtered through 0.22 micro sterile filter units (Nalgene) at 4°C over two days. Retentate was scraped off filter membranes and resuspended with 50 mM NaAc buffer, pH 5.5, to the original synovial fluid volume containing proteolytic inhibitors: 1 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM parachloromercuricbenzoic acid (PCMB), and 10 mM ethylenediamine tetraacetate (EDTA). Digestion of hyaluronic acid was carried out at 37°C by streptomyces hyaluronidase at 1 U/ml of resuspended synovial fluid. The digest was loaded on a DEAE column (Whatman International, Maidstone, UK) settled volume of 300 ml, equilibrated with NaAc buffer, 50 mM and washed with 1.5 L of the same buffer. The material with lubricating activity was eluted off of the DEAE matrix with 1 M NaCl. A 1 L wash was collected and concentrated via a 500 ml Amicon flow cell with an XM-100 membrane (mw cutoff 100 kDa). The concentrated sample was dialyzed against 25 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.5 mM $CaCl_2$.

The DEAE-bound concentrate was loaded onto a peanut agglutinin (PNA)-agarose affinity column with a settled bed volume of 25 ml, equilibrated at room temperature with 25 mM phosphate and 0.15 NaCl buffer, pH 7.4. Unbound protein was eluted with the same buffer until absorbance at 230 and 280 nm decreased to background. Material with lubricating activity

was maximally eluted in the presence of a step-wise gradient of α-lactose at a concentration of 0.07 M in 25 mM Tris and 0.15 M NaCl at pH 7.4. This material was loaded onto an Actigel ALD agarose (Sterogene Bioseparations, Arcadia, CA) coupled via amine groups to a murine monoclonal antibody against human fibronectin (Zymed Laboratories Inc., San Francisco, CA) to remove fibronectin as a contaminant. Eluted material was assayed for purity on SDS-PAGE (5-15% acrylamide) stained with Coomassie blue and by HPLC.

Protein electrophoresis standards were from GibcoBRL (Grand Island, NY), and DNA ladder standard was from FMC Bioproducts (Rockland, ME).

High Pressure Liquid Chromatography

A Bondpak C18 3.9 x 150 mm column (Waters, Milford, MA) was eluted in reverse phase with 45% (v/v) methanol (Sigma) and 5% (v/v) acetonitrile (Aldrich) HPLC grade at 1 ml/min at 35°C. The eluate was assayed by a photo diode array detector PDA 996 (Waters), and material in peak fractions were analyzed by purity plots calculated using Millenium 32 software (Waters).

Friction Apparatus

A standard friction apparatus (e.g., an apparatus described by Jay et al., 1992, Conn. Tiss. Res. 28:71-88 or Jay et al., 1998, J. Biomed. Mater. Res. 40:414-418) was used to measure lubricating activity. Natural latex was oscillated against a ring of polished glass with a constant contact area of 1.59 cm^2. The bearing system was axially loaded within a gimbals system free to rotate around two perpendicular horizontal axes. Latex and glass as bearing materials were chosen because they offer a flat surface with small asperity heights on the order of 0.05 mm. Latex, like cartilage, is compliant. Within the gimbals system, these surfaces possess near perfect co-planarity. Accordingly, fluid wedges were not generated and only a thin layer of boundary fluid was present. The entraining velocity (i.e., sliding speed) was 0.37 mm/sec with a constant contact pressure of 0.35×10^6 N/m^2.

The friction apparatus recorded displacements of the gimbals system around the vertical loading axis through a linear displacement voltage transducer, the output voltage of which was directly proportional to the magnitude of the frictional torque. The peak to peak amplitude of this signal was related to μ by a previous calibration with known frictional torque.

Test surfaces were cleaned extensively before use. A 3.8 x 3.8 cm piece of latex strapped onto the stainless steel stud was washed under running distilled deionized water (DDW) for 2

mm. It was then placed in a shallow bath of 0.9% NaCl physiological saline (PS). The glass slide was scrubbed with a 1% (v/v) 7x detergent (Flow Laboratories, McLean, VA) solution in DDW for 10 mm and then allowed to soak in the same solution at 100°C. A 5 min. scrubbing was also performed with the hot 7X solution followed by rinsing for 2-4 mm. under running DDW.

The μ was measured at 35°C and was preceded by a baseline measurement of the μ with PS. Lubrication was manifested by a reduction of μ relative to the μ of PS. Negative delta values indicate lubrication, whereas positive values indicate friction. Addition of 200 μl of PS and later 200 μ1 of test lubricant was followed by bringing the bearing surfaces close enough so that the solution wet both surfaces. After 5 mm for equilibration, the latex-coated bearing was brought to rest on the glass as it was oscillating. Peak to peak voltages were automatically recorded after 1, 3 and 5 mins. At this point, the surfaces were separated for 2 min. and then brought back together for another 5 mm session. The 3 and 5 min. μ values of the last two 5 min. sessions typically stabilized and were recorded.

Human serum fibronectin was purchased from Sigma Chemical (F0895, St. Louis, MO) and dialyzed against PS before use in the friction apparatus.

Boundary lubricants exert their effect by changing the physico-chemical characteristics of a surface. Bearing surfaces must generate a mutual repulsion in order to be lubricated in the boundary mode. Typical room temperature examples of boundary lubricants are graphite, teflon and molybdenum sulphide. Such compositions reduce friction between bearing surfaces, and therefore, are used as positive controls in assay to measure the lubricating properties of tribonectins. Tribonectins are boundary lubricants that can have an amphipathic character by coating non-biologic hydrophobic surfaces such as latex. The oligosaccharide component of a tribonectin networks with the surrounding aqueous environment. When the ultimate and penultimate sugars are removed from a naturally-occurring tribonectin purified from synovial fluid, the lubricating ability is eliminated.

The latex:glass arthrotripsometer offers an expedient way to test purified biological lubricating factors repetitively with reproducibility. Natural latex and polished glass represent bearing surfaces with little if any variation in physico-chemical characteristic from test to test. By contrast, resected cartilage apposed to either polished glass or cartilage itself will experience deformation that cannot be accurately controlled. The μ observed in a cartilage-cartilage bearing

lubricated by synovial fluid was between 0.005 and 0.024. The values of μ in the latex:glass system were appreciably higher and typically 0.04 or less. Differences in μ between the bearing materials are attributed to the 80% (w/w) water content of cartilage.

Protein Sequencing by Liquid Chromatography and Mass Spectrometry (LCMS)

Standard LCMS was carried out on tryptic digests of the purified lubricating material described above. Excised bands from 2 mm thick 5-20% gradient SDS-PAGE gels (Bio-rad Laboratories, Hercules, CA) containing the lubricating material was analyzed. The material was deglycosylated by NaNase III and O-glycosidase DS (Glyko, Novato; CA). Deglycosylation was carried out with the above enzymes at activities of 0.17 U/ml and 0.10 U/ml, respectively, for 18 hrs in the presence of 0.5 mg/ml of a tribonectin purified from synovial fluid. In all cases, the gel slices were cut through the middle of the band and were 16 mm^3 in size. All contact surfaces were carefully cleaned with 50% (v/v) acetonitrile. Sequence data was entered into the BLAST GENBANK® search algorithm and matches identified.

Isolation and Culture of Human Synovial Fibroblasts

Human synovium with a normal appearance was obtained from a 30 year old white male undergoing arthroscopy. Within 1 hr after surgery, the synovial tissue explant was washed three times with Dulbecco's calcium- and magnesium-free phosphate-buffered saline (GIBCO). Pieces 2 mm^3 in size were placed in Dulbecco's modification of Eagle's medium (GIBCO), supplemented with 100 U of penicillin and 100 μg of streptomycin per ml (GIBCO), containing 4 mg/ml of Clostridiopeptidase A (Worthington Biochemical CLS, 125-200 U/mg) sterilized through a 0.22 mm filter (Nalge). The tissue fragments were further divided with scissors in a 100 mm plastic petri dish (Falcon) and incubated for 4 hrs in 20 ml of medium at 37°C in a moist atmosphere of 5% carbon dioxide and 95% air.

The digest was well mixed many times by aspiration S into and expulsion from a Pasteur pipette. An equal volume of 0.05% trypsin and 0.02% EDTA in modified Puck's Saline A (GIBCO) were added and incubation continued for a further hour under the same conditions. The suspension was centrifuged 10 min at 400 x g at 23°C and washed three times each with 40 ml of calcium- and magnesium-free phosphate-buffered saline. The pellet was suspended in modified Eagle's medium (20 ml) supplemented with 10% fetal bovine serum (Flow Laboratories), 100 U of penicillin, and 100 mg of streptomycin per ml. Two milliliters of this final mixture were plated per 60 mm plastic petri dish (Falcon). Synovial fibroblasts were grown

to confluence and cells harvested. Human skin fibroblasts (American Type Culture Collection (ATCC) Designation CCD-10995K; ATCC, Mannassas, VA) which served as a control were also grown and harvested using the procedure described above.

RNA Extraction and RT-PCR Analyses

RNA from synovial and skin fibroblasts was purified by RNeasy mini-columns and reagents (Qiagen, Crawley, Ltd., UK). Contaminating genomic DNA was removed by DNAshredder and DNase (RNase free) (Qiagen). First strand cDNA was synthesized by reverse transcription and PCR amplification using the following oligonucleotide primers. MSF-exon 6 forward primer 5' -CCAAACCACCAGTTGTAGATGAAGC-3' (SEQ ID NO:15) and MSF-exon 6 reverse primer-GCGGAAGTAGTCTTCTCTTTTCCATCAG3' (SEQ ID NO:16). These primers correspond to nucleotide position numbers 674-698 and 953-926, respectively, of the human MSF gene (SEQ ID NO:2; GENBANK® accession number U70136). Thermal cycling conditions were 42°C for 12 mins., 95°C for 10 mins., followed by 43 cycles between 94°C x 20 secs and 55-65°C x 30-90 secs. A final extension for 7 mins was at 72°C (Perkin Elmer Biosystems).

Alternative splice variant of MSF is a Tribonectin

A lubricating polypeptide was purified from human synovial fluid using standard biochemical methods followed by affinity chromatography with peanut agglutinin. The final fraction, which solely possessed lubricating ability, contained a product with an apparent molecular weight of 280 kDa. Components with a molecular weight in excess of 280 kDa were not observed. LCMS performed on tryptic fragments from the 280 kDa excised band indicated the presence of two different proteins that matched in the BLAST search algorithm to fibronectin precursor and MSF (GENBANK™ Accession No. U70136). Sequences of MSF were identified from both native and deglycosylated lubricating polypeptides. Accordingly, the purification scheme was terminated with an anti-fibronectin column resulting in the elimination of fibronectin as an impurity (as assayed by C18 analytical HPLC and purity plot analysis). In addition, lower molecular weight bands at 70 and 160 kDa on SDS-PAGE were absent from the purified tribonectin preparation eluting -from the anti-fibronectin column. The purified tribonectin assayed in the friction apparatus was found to display boundary lubricating activity similar to that of whole synovial fluid (Table 5). By contrast, purified serum fibronectin raised friction indicating that synovial fluid lubricating ability was mediated by the purified tribonectin.

Table 5: Friction coefficients for a tribonectin purified from human synovial fluid and fibronectin (Mean +SD; N=3)

LUBRICANT*		(PS**)	
Tribonectin	0.047 ± .006	0.131 ± .007	-0.084 ± .004
HSF†	0.040 ± .005	0.135 ± .009	-0.095 ± .011
Fibronectin	0.181	0.136	+0.045 ± .005

* Tested at a concentration of 250 μg/ml in PS.
** Physiological saline.
† Post-mortem human synovial fluid

Furthermore, LCMS of tryptic fragments identified portions of exons 6 through 9 of MSF, inclusively. Purified tribonectin reacted to peanut agglutinin indicating the presence of beta (l-3) Gal-GalNAC oligosaccharides by virtue of its purification. An increase in electrophoretic mobility was observed after digestion with NaNase III and O-glycosidase DS, indicating that the purified tribonectin is highly glycosylated via O-linked oligosaccharides. The apparent molecular weight of deglycosylated tribonectin purified from synovial fluid was 120 kDa.

RT-PCR analysis was completed using primers specific for nucleotide sequences encoding the N-terminal end of exon 6 of MSF. RT-PCR's using human synovial fibroblast RNA generated a 280 bp product, the predicted distance between the designed primers. Similar experiments without reverse transcriptase did not generate this product indicating that the RNA was free of genomic DNA. Purified RNA from skin fibroblasts did not produce any product using the same primers.

MSF was first isolated from human monocytes; a 25 kDa fragment of MSF was found to stimulate the development of megakaryocytes. MSF precursor protein is 1404 residues in size and constructed from 12 exons. Exon 6 appears to encodes a centrally located mucin that is 940 residues in length. Exon 6 has homology to vitronectin, exons 2 and 3 appear homologous to somatomedin B-like regions, and exons 8, 9 are similar to hemopexin-like regions in vitronectin. Hemopexin is a serum heme scavenging protein that interacts with hyaluronate.

A tribonectin purified from synovial fluid and an articular cartilage superficial zone protein (SZP) purified from articular cartilage share sequence identity with MSF but differ in their apparent molecular weights and amino acid sequences.

Example 1: MSF Gene Expression by Human Synovial Fibroblasts

A naturally-occurring tribonectin was found to be expressed by human synovial fibroblasts from a gene encoding MSF. Some, but not all, of the exons of the MSF gene are represented in the tribonectin gene product. For example, the tribonectin contains sequences encoded by exons 6-9 of the MSF gene, but lacks sequences encoded by at least one exon of the naturally-occurring MSF gene.

MSF gene expression was evaluated as follows. Gel slices from the 240 kDa apparent molecular weight band of purified tribonectin from pooled human synovial fluids with normal lubricating ability were digested with trypsin. N-terminal sequencing of tryptic fragments was performed by liquid chromatography mass spectrometry and results compared to known sequences in GenBank™. Digestion of purified tribonectin with O-glycosidase DS and NaNaseIII was used to indicate the molecular weight of the core protein. Lubricating ability was assayed using a standard friction apparatus that oscillates natural latex against a ring of polished glass.

Two previously identified protein species were present in the final lubricating fraction of human synovial fluid. Tryptic fragments from fibronectin precursor (FN) and megakaryocyte stimulating factor (MSF) were both identified. A 100% match (E values range 0.31 – 0.047) was observed with MSF sequences specific for exons 6 though 9. MSF is 1104 amino acids in size with multiple functional domains- similar to vitronectin. This tribonectin reduced the coefficient of friction (μ) from 0.142 to 0.036 whereas purified serum fibronectin raised μ from 0.136 to 0.181. Forward and reverse RT-PCR primers corresponding to amino acid positions 214 – 222 and 300 – 309 of SEQ ID NO:1 were used to probe purified mRNA from human synovial fibroblasts grown *in vitro*. A 280 bp gene product was observed consistent with the predicted amino acid sequence. The data described herein indicate that a naturally-occurring tribonectin is secreted by synovial fibroblasts via expression of the MSF gene.

The methods described below were used to characterized a lubricating component in synovial fluid.

Human Synovial Fluid Collection

Aliquots of synovial fluid from patients undergoing diagnostic arthroscopy and total knee replacement were collected and assayed in the friction apparatus. In both cases, the synovial fluid was aspirated prior to initiation of any surgical procedure and immediately centrifuged at 10,000 x g at 4°C for 2 hrs to remove cellular debris. Samples that were grossly contaminated with blood were discarded. Aliquots with normal lubricating ability were pooled and stored at -20°C.

Purification and Isolation of Human Tribonectins

Human synovial fluid 200 ml was filtered through 0.22 μm sterile filter units (Nalgene) at 4°C over two days. Retentate was scraped off filter membranes and resuspended with 50 mM NaAc buffer, pH 5.5, to the original synovial fluid volume. The resuspension buffer contained proteolytic inhibitors: 1mM PMSF, 1 mM PCMB and 10 mM EDTA. Digestion of hyaluronic acid was carried out at 37°C by streptomyces hyaluronidase at 1 U/ml of resuspended synovial fluid. The digest was loaded on a DEAE column (Whatman International, Maidstone, UK) settled volume of 300 ml, equilibrated with NaAc buffer, 50 mM and washed with 1.5 L of the same buffer. The desired material was eluted from the DEAE matrix with 1 M NaCl. A 1 L wash was collected and concentrated via a 500 ml Amicon flow cell with an XM-100 membrane (mw cutof = 100 kDa). The concentrated sample was dialyzed against 25 mM phosphate buffer, pH 7.4, containing 0.15 M NaCl and 0.5 mM $CaCl_2$.

The DEAE-bound concentrate was loaded onto a peanut agglutinin (PNA)-agarose affinity column with a settled bed volume of 25 ml, equilibrated at room temperature with 25 mM phosphate and 0.15 NaCl buffer, pH 7.4. Unbound protein was eluted with the same buffer until absorbance at 230 and 280 nm decreased to background. Desired material was maximally eluted in the presence of a step-wise gradient of α-lactose at a concentration of 0.07 M in 25 mM Tris and 0.15 M NaCl at pH 7.4. Pre-purified tribonectin was loaded onto an Actigel ALD agarose (Sterogene Bioseparations, Arcadia, CA) coupled via amine groups to a murine monoclonal antibody against human fibronectin (Zymed Laboratories Inc., San Francisco, CA). Eluted material was assayed on SDS-PAGE 5-15% stained with Coomassie blue and by HPLC.

High Pressure Liquid Chromatography

A μBondapak C18 3.9 x 150 mm column (Waters, Milford, MA) was eluted in reverse phase with 45% (v/v) methanol (Sigma) and 5% (v/v) acetonitrile (Aldrich) HPLC grade at 1 ml/min at 35°C. Eluate was assayed by a photo diode array detector PDA 996 (Waters) and peaks analyzed by purity plots calculated using Millenium 32 software (Waters).

Friction Apparatus

A standard friction apparatus was used to measure lubricating activity as described above

The μ was measured at 35°C and was preceded by a baseline measurement of the μ with PS. Lubrication was manifested by a reduction of μ relative to the μ of PS. Negative Δμ values indicate lubrication whereas positive values indicate friction. Addition of 200 μl of PS and later 200 μl of test lubricant was followed by bringing the bearing surfaces close enough so that the solution wet both surfaces. After 5 min for equilibration, the latex-coated bearing was brought to rest on the glass as it was oscillating. Peak to peak voltages were automatically recorded after 1, 3 and 5 mins. At this point the surfaces were separated for 2 min and then brought back together for another 5 min session. The 3 and 5 min μ values of the last two 5 min sessions typically stabilized and were recorded.

LCMS

LCMS of tryptic digests was carried out as described above. Bands were excised from 2 mm thick 5-20% gradient SDS-PAGE gels (Bio-rad Laboratories, Hercules, CA) for further analysis. Deglycosylation was carried out using NaNase III and O-glycosidase DS (Glyko, Novato, CA) (at concentrations of 0.17 U/ml and 0.10 U/ml, respectively, for 6 hrs in the presence of 0.5 mg/ml of protein. In all cases, the gel slices were cut through the heart of the band and were 16 mm^3 in size. All contact surfaces were carefully cleaned with 50% (v/v) acetonitrile. Sequence data was entered into the BLAST GenBank™ search algorithm and matches identified.

RNA Extraction and RT-PCR Analyses

RNA from synovial and skin fibroblasts was purified by RNeasy mini-columns and reagents (Qiagen, Crawley, Ltd., UK). Contaminating genomic DNA was removed by DNAshredder and DNase (RNase free) (Qiagen). First strand cDNA was synthesized by reverse transcription and PCR amplification using the following oligonucleotide primers (the relative

positions of the MSF-specific primers are shown in Fig. 1): MSF-exon 6 forward primer 5'-CCAAACCACCAGTTGTAGATGAAGC-3' (SEQ ID NO:15) and MSF-exon 6 reverse primer 5'-GCGGAAGTAGTCTTCTCTTTTCCATCAG-3' (SEQ ID NO:16). These primers correspond to base pair position numbers 674-698 and 953-926 respectively in GENBANK™ Acession number U70136 (human MSF). Thermal cycling conditions were 42°C for 12 mins, 95°C for 10 mins followed by 43 cycles between 94°C × 20 secs and 55 – 65°C × 30 – 90 secs. A final extension for 7 mins was at 72°C (Perkin Elmer Biosystems).

Miscellaneous Reagents

Human serum fibronectin was purchased from Sigma Chemical (F0895, St. Louis, MO) and dialyzed against PS before use in the friction apparatus. Protein electrophoresis standards were from GibcoBRL (Grand Island, NY) and DNA ladder standard was from FMC Bioproducts (Rockland, ME).

A tribonectin from from human synovial fluid was purified. The final fraction which solely possessed lubricating ability primarily contained a product with an apparent molecular weight of 280 kDa. The fraction also contained minor low molecular weight components, which were removed with the final antibody-based purification step. Components with a molecular weight in excess of 280 kDa were not observed. LCMS performed on tryptic fragments from the 280 kDa excised band indicated the presence of two different proteins that matched in the BLAST search algorithm to fibronectin precursor and MSF (GENBANK™ Accession No. U70136). Sequences of MSF were identified from both native and deglycosylated tribonectin. Accordingly, the purification scheme was terminated with an anti-fibronectin column resulting in the near elimination of fibronectin as an impurity on the basis of C18 analytical HPLC and purity plot analysis. In addition, the minor low molecular weight bands at 70 and 160 kDa on SDS-PAGE were absent from the purified preparation eluting from the anti-fibronectin column. Purified tribonectin assayed in the friction apparatus was found to display boundary lubricating activity similar to that of whole synovial fluid (Table 6).

Table 6: Friction coefficient for a human tribonectin and fibronectin

LUBRICANT†		(PS*)	
Tribonectin	0.035	0.142	-0.106
Fibronectin	0.181	0.136	+0.045

* Physiological saline.

† Tested at a concentration of 250 μg/ml in PS.

In contrast, purified serum fibronectin raised friction indicating that synovial fluid lubricating ability was mediated by tribonectin.

LCMS of tryptic fragments correctly identified portions of exons 6 through 9 of MSF inclusively. Fragments encoded by nucleic acid sequences at the beginning and end of exon 6 were found. The tribonectin reacted to PNA indicating the presence of β(1-3)Gal-GalNAC oligosaccharides by virtue of its purification. In addition there was a very significant increase in electrophoretic mobility after digestion with NaNase III and O-glycosidase DS indicating that the tribonectin is highly glycosylated via O-linked oligosaccharides. The apparent molecular weight of deglycosylated tribonectin was 120 kDa.

RT-PCR analysis was completed using primers specific for nucleotide sequences encoding the N-terminal end of exon 6 of MSF. RT-PCR's using human synovial fibroblast RNA generated a 280 bp product, the predicted distance between the designed primers. Similar experiments without reverse transcriptase did not generate this product indicating that the RNA was free of genomic DNA. Purified RNA from skin fibroblasts did not produce any product using the same primers.

The data described herein indicate that the exons of the MSF gene are alternatively spliced to express lubricating polypeptides in synovial joints. Synovial fibroblasts are one of the the sources of such tribonectins in joints. An isolated naturally-occurring tribonectin described herein (Mr~280 kDa) and SZP (Mr~345 kDa) are different molecules as they differ in apparent molecular weight by 65 kDa. It is possible that MSF is a precursor for both molecules by virtue of differing expression of MSF exons by two independent cell lines that populate the synovial cavity. Repetitive LCMS sequencing of tryptic digests did not identify any of the exons which bracket exons 6 through 9 in MSF. A naturally-occurring tribonectin purified from bovine calf was found to have the same molecular weight as a tribonectin purified from the human sources.

Boundary lubricants exert their effect by changing the physico-chemical characteristics of a surface. Bearing surfaces must generate a mutual repulsion in order to be lubricated in the boundary mode. Commonplace room temperature examples are graphite, teflon and molybdenum sulphide. Tribonectins appear to have an amphipathic character by coating non-biologic hydrophobic surfaces such as latex. The extensive glycosylations are important to

network with the surrounding aqueous environment. When the ultimate and penultimate sugars are removed the lubricating ability is eliminated. The possibility of hyaluronate binding to SZP or a tribonectin via protein expressed by exon 9 supports a mechanism by which hyaluronate plays a role in synovial fluid. A hyaluronate polymer may serve to bind many SZP or tribonectin molecules in tandem side-by-side, thus distributing shear stress and stabilizing lubricant molecules.

The primary structure of MSF precursor (alternatively spliced gene products such as tribonectins) is different from that of MG2, statherine, and proline rich glycoprotein which are found in saliva and lubricate dental bearings. Only MG2 has been tested in the present latex:glass bearing and found to lubricate as effectively as a tribonectin. Both glycoproteins share the same O-linked β(1-3)Gal-GalNAc moiety. These data indicate that the O-linked β(1-3)Gal-GalNAc moiety is important for boundary lubrication activity.

Enzymatic digestion experiments indicate that boundary lubrication provided by synovial fluid is highly sensitive to trypsin. These experiments demonstrated that the removal of lubricating ability by phospholipases (contaminated with very small amounts of trypsin-like proteases) could be eliminated by co-incubation with protease inhibitors, leupeptin and aprotinin. Lysine and arginine comprises 13.5% and 1.3% respectively of the exon 6 product which supports the observation of trypsin sensitivity, even though this is the same region of tribonectin that is extensively glycosylated and could therefore prevent proteolysis. It is likely that even minor inflammatory states that elaborate trypsin or elastase have deleterious effects upon the lubricating ability of naturally-occurring tribonectins. Non-lubricated cartilaginous bearings may experience premature wear which can initiate osteoarthritis. Such conditions are treated using the lubricating polypeptides, i.e., tribonectins, described herein.

Example 2: Homology of a Tribonectin and SZP: Products of MSF Gene Expression by Human Synovial Fibroblasts and Articular Chondrocytes Localized to Chromosome 1q25

Expression of tribonectins was evaluated in primary human synovial fibroblasts and primary articular cartilage. RNA was purified from human synovial fibroblasts and articular chondrocytes grown *in vitro* from tissue explants obtained from subjects without degenerative joint disease. RT-PCR was used with multiple complimentary primer pairs spanning the central mucin expressing exon 6 of the MSF gene and individual exons on both the N- and C-terminal sides of exon 6. Exons 2, 4 and 5 appear to be variably expressed by synovial fibroblasts and

articular chondrocytes. A lubricating polypeptide, a tribonectin, expressed from various exons of the MSF gene is expressed by both chondrocytes and synovial fibroblasts *in vitro*. Both a trobonectin and superficial zone protein (SZP), a related proteoglycan, share a similar (but not identical) primary structure. Tribonectins and SZP also differ in post-translational modifications with O-linked oligosaccharides; tribonectins contain O-linked oligosaccharides, whereas such post-translational modifications have not been detected in SZP. The oligosaccharides are predominant in tribonectins; limited amounts of chondroitin and keratan sulfate are found in SZP. Since most of the MSF exons are involved in the expression of a tribonectin, a strong homology to vitronectin persists. Screening of a human genome BAC library with a cDNA primer pair complimentary for exon 6 identified two clones. Both clones were complimentary for chromosome 1q25 by *in situ* hybridization. This locus was implicated in camptodactyl-arthropathy-pericarditis syndrome (CAP) by genetic mapping. CAP, a large joint arthropathy, is associated with ineffective boundary lubrication provided by synovial fluid. Accordingly, the tribonectins described herein are useful to prevent and/or treat CAP.

The data described below addresses the following questions: 1) Which of the 12 MSF exons are expressed by synovial fibroblasts and presumably result in tribonectin secretion into the synovial cavity, 2) how does this differ from the expression of MSF by articular chondrocytes which results in SZP secretion onto the surface of cartilage, 3) do tribonectins and SZP share the same primary structure, 4) are tribonectins post-translationally modified with chondroitin sulfate- a feature of SZP, and 5) does an isolated exon 6 product possess boundary lubricating ability.

Human synovial fluid was collected and processed as described above, and tribonectins were purified and isolated from human tissue using methods described above. Lubricating activity was measured using a standard friction apparatus.

Immunodetection of Chondroitin-6-Sulfate

Monoclonal antibody M621C (ICN, Aurora, OH) was use to detect the presence of chondroitin-6-sulfate in purified tribonectin. Standard dot and Western blotting techniques were used.

Digestion of a tribonectin with Chymotrypsin

TLCK-treated chymotrypsin 0.1BTEE U/ml of human synovial fluid was incubated for 1 hr at 37°C. Immediately following digestion lubricating activity was assayed in the friction

apparatus. Additional digestions were carried out in the same way but also in the presence of 10 μg/ml leupeptin and 5 μg/ml aprotinin in the event that commercial TLCK treatment incompletely inactivated contaminating trypsin.

Isolation and Culture of Human Articular Chondrocytes

In addition to isolating human synovial fibroblasts as described above, human chondrocytes were isolated. Cartilage from the femoral condyles and tibial plateaus of human knee joints was obtained at autopsy from donors without known history of joint disease or from healthy organ donors from tissue banks. Cartilage slices were cut into 2-3mm^3 pieces, washed with DMEM (Whittaker MAB Bioproducts, Walkerville, MD) and treated for 15 min with trypsin 10% (v/v) in a 37°C waterbath.. The tissues were transferred to DMEM, 5% FBS, penicillin-streptomycin-fungizone, and 2 mg/ml *C. perfringens* collagenase type IV (Sigma) and digested overnight on a gyratory shaker. The cells were washed 3 times with DMEM and cultured in DMEM containing 5% FBS.

Culture of Human Monocytes

Human monocytes CRL-9855 in suspenson were purchased from American Type Culture Collection (Mannassas, VA). Cells were concentrated by centrifugation and then resuspended in a T75 flask to concentration between 0.2 and 1.0 x 10^6/ml with Iscove's modified Dulbecco's medium supplemented with 10% fetal bovine serum that was not heat inactivated (Flow Laboratories), 0.03 mM thymidine, 0.1 mM hypoxanthine and 0.05 mM 2-mercaptoethanol. As the monocytes multiplied, the culture was expanded to maintain the above concentration range.

RNA Extraction and RT-PCR Analyses

RNA from human synovial and articular chondrocytes and monocytes were purified by RNeasy mini-columns and reagents (Qiagen, Valencia,CA). Contaminating genomic DNA was removed by DNAshredder and DNase (RNase free) (Qiagen). Complimentry DNA synthesis was done by incubating at 25°C for 10 min for extension of hexameric primers by reverse transcriptase, and synthesis of cDNA at 42°C for 12 min, and activation of Ampli Taq/denaturation of RNA-cDNA complex at 95C for 10 min. Thermal cycling condition was 43 cycles of 94°C, 20 secs for denaturation and 62°C, 60 secs, and a final extension at 72°C for 7 mins (Perkin Elmer Biosystems, Foster City, CA). RT-PCR products were analyzed by 3% NuSieve GTG agarose (FMC, Rockland, ME) gel electrophoresis stained with ethidium bromide.

Bands not corresponding to expected RT-PCR product sizes were excised and extracted from agarose (Qiagen).

BAC clone library screening

An *E. coli* BAC library containing the human genome was screened (Research Genetics Inc., Huntsville, AL) with a cDNA primer pair complimentary to MSF exon 6 by PCR. Forward primer 5'-CCAAACCACCAGTTGTAGATGAAGC-3' (SEQ ID NO:15) and reverse primer 5'-GCGGAAGTAGTCTTCTCTTTTCCATCAG-3' (SEQ ID NO:16) complimentary to base pair position numbers 674-698 and 953-926 of SEQ ID NO:2. Candidate *E. coli* clones were grown in LB agar containing 12.5 mg/ml chloramphenicol at 37°C for 16 hrs. Large construct DNA free of genomic DNA was purified by anion exchange and exonuclease digestion (Qiagen Large-construct kit, Valencia, CA).

In situ hybridization of BAC clone DNA and chomosomes

Metaphase chromosomes were prepared from short term lymphocyte cultures from a normal individual according to established protocols. Two micrograms of BAC DNA from positive clones were labeled using BioNick labeling kit (Life Technologies, MD) using biotin 14-dATP in a nick translation reaction according to a protocol supplied by the manufacturer. After nick translation, the reaction products were purified on a G-50 column. 100ng of labeled DNA and 20μg of cot1 DNA were co-precipitated with ethanol. The DNA was recovered by centrifugation and dissolved in 20μl of hybridization solution (Hybrisol vii, Oncor). Probe hybridization, washing, microscopy and mapping of the probes were performed according to standard procedures.

Digestion of Tribonectins with Chymotrypsin and Assay for Lubricating Ability

A tribonectin was purified from pooled human synovial fluid with normal lubricating ability defined as $\Delta\mu < -0.06$ and possessed an apparent molecular weight of 280 kDa. Terminating the purification with an anti-fibronectin step was carried out to remove contaminating bands. This step also reduced the purity angle to < 6°. A tribonectin at a concentration of 250 μg/ml lubricated the latex:glass bearing as effectively as post-mortem synovial fluid from a subject without degenerative joint disease (Table 7).

Table 7. Friction coefficients for human a human tribonectin and fibronectin

Lubricant	μ	μ (PS‡)	Δμ	N
Tribonectin†	0.047 ± .006	0.131 ± .007	-0.084 ± .004	3
HSF*	0.040 ± .005	0.135 ± .009	-0.095 ± .011	3
Chmyotrypsin§	0.130 ± .041	0.107 ± .024	+0.02 ± .035	3

Mean ± SD
†Tested at a concentration of 250 μg/ml in PS.
‡Physiological saline
*Post-mortem human synovial fluid
§HSF digested with TLCK-treated chymotrypsin in the presence of leupeptin and aprotinin.

Enzymatic digestions with TLCK-treated chymotrypsin were performed to determine if the isolated exon 6 protein product possesses lubricating ability as this domain does not contain aromatic amino acids. The resulting digestate raised μ to .130 from the saline control value μ = .107. Similar digestions in the presence of leupeptin/aprotinin continued to demonstrate no lubricating ability (Δμ= +.02).

Immunodetection of Chondrotin-6-Sulfate

Monoclonal antibody M621C was used to probe tribonectins transferred to nitrocellulose. No reaction was observed corresponding to the 280 kDa band on the original SDS-PAGE. This indicates that tribonectins do not contain chondroitin-6-sulfate.

RT-PCR Analysis of Synovial Fibroblast and Articular Chondrocyte RNA

RT-PCR of chondrocyte and synovial fibroblast mRNA was carried out using the primers illustrated in Table 8. RT-PCR products of the predicted size in addition to others that were smaller than predicted were observed for both cell lines. The primer pair spanning the C-terminal end of exon 6 to the N-terminal end of exon 12 was observed to be 769bp, its predicted length, for both cell lines. Similarly, the primer pair spanning the same position in exon 6 to exon 11 produced an expected RT-PCR product of 654bp. The primer pair of exon 5 and 6 produced the expected RT-PCR product of 278bp for both cell lines.

Table 8: Primer pairs used in RT-PCR's with RNA from human synovial fibroblasts and articular chondrocytes.

Inter-exon Primers	Base Pairs	(5' – 3') Forward Primer	Base Pairs	(5' – 3') Backward Primer
1 – 6	64 – 85	TTGTTGCTGCTGTCTGTT TTCG (SEQ ID NO:17)	819 – 796	GGGTCTGGGATTTATTGGTTTTGC (SEQ ID NO:23)
2 – 6	128 – 149	GGAGATGTGGGGAAGG GTATTC (SEQ ID NO:18)	819 – 796	GGGTCTGGGATTTATTGGTTTTGC (SEQ ID NO:24)
4 – 6	371 – 394	CACCATCTTCAAAGAAA GCACCTC (SEQ ID NO:19)	819 – 796	GGGTCTGGGATTTATTGGTTTTGC (SEQ ID NO:25)
5 – 6	542 – 566	CCTCCTCTTCCTCTTCTT CTTCAAC (SEQ ID NO:20)	819 – 796	GGGTCTGGGATTTATTGGTTTTGC (SEQ ID NO:26)
6 – 11	3427 – 3448	GGCATTATCATCAATCC CATGC (SEQ ID NO:21)	4080 – 4057	CACATTTGGAAGTCCTCTCCACAG (SEQ ID NO:27)
6 – 12	3427 – 3448	GGCATTATCATCAATCC CATGC (SEQ ID NO:22)	4195 – 4171	TTGCTCTTGCTGTTCTACTAGGCAC (SEQ ID NO:28)

Base pair position numbers correspond to accession number U 70136 in GENBANK ™.

Table 9:. Primer pairs used in RT-PCR's with RNA from human monocytes.

Inter-exon Primers	Base Pairs	(5' – 3') Forward Primer	Base Pairs	(5' – 3') Backward Primer
1 – 3	64 - 85	TTGTTGCTGCTGTCTGTTTT CG (SEQ ID NO:29)	313 - 291	CATACTTCTTACATTGGGCG TCG (SEQ ID NO:32)
1 – 4	64 - 85	TTGTTGCTGCTGTCTGTTTT CG (SEQ ID NO:30)	375 – 354	TGGTGGTGATGTGGGATTAT GC (SEQ ID NO:33)
2 – 5	128 - 149	GGAGATGTGGGGAAGGGT ATTC (SEQ ID NO:31)	540 - 517	GGAGGAGGAGGACTCTTGA TTTTC (SEQ ID NO:34)

Base pair position numbers correspond to accession number U 70136 in GENBANK™.

The primer pair spanning from exon 1 to exon 6 failed to singularly generate the predicted product size of 756 bp. Instead three smaller sized (350, 450 and 490bp) bands were observed for both cell lines. The primer pair spanning from exon 2 to the exon 6 N-terminus produced a product roughly 420bp in size for both cell lines and was 272bp (692bp – 420bp) shorter than its predicted size. The predicted RT-PCR 692 bp product was faintly observed for chondrocytes and less so for synovial fibroblasts. The exon 2 to 6 primer pair 420bp product was excised and sequenced which revealed the absence of exon 5 and most of exon 4 except for the first two N-terminal amino acids Ala[105] and Leu[106] of SEQ ID NO:1) Sequencing of the 490 and 450bp RT-PCR products from the exon 1 to 6 primer pair identified the same deletions.

Sequencing of the smaller 350bp product from the exon 1 to 6 primer pair revealed these same deletions in addition to exon 2 except for the N-terminal Q^{25}. The primer pair of exon 4 and 6 produced the expected RT-PCR product of 449bp for both cell lines and a smaller 340bp product. Sequencing of this 340bp product revealed deletion of exon 5 except for the N-terminal E^{156}.

Alternative splicing was found to be responsible for generating 4 different phenotypical isoforms of MSF from both synovial fibroblasts and chondrocytes (Figs. 2A and 2B).

Phenotype V0 consists of all 12 exons, V1 missing exon 5, V2 missing exon 5 and most of 4, and V3 missing exon 5 and most of 4 and 2. Yet another isoform is a polypeptide which contisn amino acids encoded by MSF exons 1 and 6-12 (missing exon 5); this is likely the smallest naturally-occurring tribonectin. Each isoform contains the lubricating domain exon 6. The domain encoded by exon 1 plays a role in mediating binding of the tribonectin to hydrophobic surfaces like cartilage

RT-PCR Analysis of Monocyte RNA

RT-PCRs with purified monocyte RNA using primer pairs (Table 9) complimentary to MSF exon 6 did not produce any products. A primer pair spanning exons 1 to 3, produced a PCR product of 250bp, its predicted size. Another primer pair spanning exons 1 to 4 produced a PCR product that was approximately 130bp smaller than the 312bp predicted size. Yet another primer pair spanning exons 2 to 5 did not generate any PCR product, indicating that exon 5 is not expressed. These data would appear to demonstrate that monocytes express exons 1 through 3, 1 through 4 variably and do not express exon 6. The forward primer sequence in exon 1 was common to all of the above RT-PCR work involving monocytes, synovial fibroblasts and chondrocytes.

In situ hybridization of BAC clone DNA and chromosomes

Screening of a human genome BAC clone library with the above MSF exon 6 cDNA primer pair resulted in 2 candidate clones. BAC clones 330 and 285 hybridized to chromosome 1 at q25 region (DAPI banding). To confirm the localization to chromosome 1, metaphase chromosomes were hybridized to BAC probes along with chromosome 1 specific centromere probe (VYSIS). Both centromeric as well as the BAC probes hybridized to chromosome 1. Subsequent G-banding of the same slides confirmed the q25 localization.

Synovial fibroblasts grown *in vitro* alternatively spliced out either MSF exons 2,4 or 5, or a combination thereof. In the case of exon 4, expression stopped after the first two codons of the exon (Ala^{105} and Leu^{106}). Isoforms without exon 5 except for residue E^{156} and one without exon 2 except for Q^{25} also exist. Three RT-PCR products occurred with the exon 1 to 6 primer pair which appear to correspond to phenotypes V1 – 3. However, the expected RT-PCR 692bp product for the exon 2-6 primer pair was observed, indicating that an isoform V0, which contains each of these exons also exists. It would appear that 4 or 5 different phenotypical isoforms of tribonectins are expressed by synovial fibroblasts. Chondrocytes share this phenotypic expression. A summary of the alternative splice sites for both cell lines is illustrated in Fig. 3. The relatively greater intensity of the 692bp exon 2-6 and 449bp exon 4-6 primer products for chondrocytes suggests that these cells could potentially select for a higher molecular weight isotype.

Exon 6 in MSF is positioned between an analogous heparin sulfate binding region (MSF exon 4) and the hemopexin-like repeats (MSF exons 8,9). Separation of these domains encourages greater interaction with glycosaminoglycan which would differ from vitronectin in which these domains are thought to compete for glycosaminoglycan binding due to their proximity. Perhaps the ability to bind to glycosaminoglycan on either or both ends of the lubicating domain serves to stabilize lubricating activity on cartilage covered with the lamina splendins. Alternatively, an interaction exists between a tribonectin and collagen type II, which interaction serves to bind the tribonectin directly to cartilage.

Boundary lubricants such as tribonectins bind to bearing surfaces and generate a mutual repulsion in order to lubricate in the boundary mode. This bifunctional attribute appears as amphipathic behavior by coating non-biologic hydrophobic surfaces such as latex. Extensive glycosylations network with the surrounding aqueous environment. When ultimate and penultimate sugars are removed the lubricating ability is eliminated. Expression of MSF exon 6 is required for lubricting activity; optimal lubricting activity likely requires the expression of a domain expressed by at least one additional MSF exon, e.g., exon 1. The chymotrypsin digestion experiment carried out was intended to excise the exon 6 product and test this digestate for lubrication assuming that other released exon products would not interfere with lubricating ability. The digestate did not lubricate we;;, despite replication in the presence of leupeptin and aprotinin, suggesting that other exon products may be needed. For example, exon 3, which has a

number of cysteine residues which are important in the aggregation of mucin polymers, may be required. The ability of a tribonectin to coat a surface is likely dependent upon hydrophobicity and the ability of individual tribonectin polymers to aggregate.

Immunohistochemical studies failed to detect chondroitin-6-sulfate indicating that it is not present or it is sterically hindered by O-linked glycosylations adjacent to $D^{220}EASG^{224}$ where chondroitin sulfate would be attached. The presence of chondroitin and keratan sulfate in SZP was confirmed by ^{35}S-labeling and release after digestion with keratanase and chondroitin lyase ABC. However, no significant change in SZP molecular weight was observed after this digestion, indicating that only a small amount of substitution was likely. It is possible that either glycosaminoglycan is conjugated to a tribonectin

Tribonectins and SZP are similar molecules sharing MSF exons 1,3 and 6 through 12, and the alternatively spliced exons 2 through 5. Despite similar primary structure, differences in post-translational modifications between the two proteins have been detected. The presence of the O-linked lubricating moiety β(1-3)Gal-GalNAc has not been detected for SZP as it has for the tribonectins described herein. A difference in molecular weight between tribonectins and SZP is supported by Western blotting with antibodies developed against SZP, which was purified from superficial zone chondrocytes.

Camptodactyl-arthropathy pericarditis syndrome (CAP)

In situ hydridization experiments indicated that DNA sequences encoding tribonectins reside on chomosome 1q25, as both BAC clone probes independently localized to the same location. A boolean search of Online Mendelian Inheritance in Man (OMIM) revealed one arthritic disease which has been genetically mapped to the same locus. Camptodactyl-arthropathy-pericarditis syndrome (CAP) is characterized by juvenile onset large joint *non-inflammatory* arthropathy and congenital non-traumatic flexion contractures of one or more inter-phalangeal joints (camptodactyl). The histopathologic appearance of synovial and teno-synovial tissues from patients with this autosomally inherited disease is that of B-type synoviocyte hyperplasia and end-stage fibrosis. CAP syndrome is a direct result of ineffective lubrication resulting in premature joint wear. Some patients with CAP also develop pericarditis. Isolated naturally-occurring or synthetic tribonectins are useful to treat CAP and for lubricating pericardial tissues. CAP is diagnosed by detecting a mutation in the MSF gene.

Other embodiments are within the following claims.